Acta Chemica Scandinavica, 1994: 48: 240–251
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904–213X

Malonofungin: an Antifungal Aminomalonic Acid from *Phaeoramularia fusimaculans*

Nina Berova,[a] Jens Breinholt,*[,b] Georg W. Jensen,[b] Anders Kjær,[c] Lee-Chiang Lo,[a] Koji Nakanishi,[a] Ruby I. Nielsen,[b] Carl E. Olsen,[d] Christian Pedersen[c] and Carsten E. Stidsen[e]

[a]Department of Chemistry, Columbia University, New York, New York 10027, USA, [b]Novo Nordisk A/S, Plant Protection Division, DK-2880 Bagsværd, Denmark, [c]Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark, [d]Chemistry Department, The Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark and [e]Novo Nordisk A/S, Bioscience, DK-2880 Bagsværd, Denmark

Berova, N., Breinholt, J., Jensen, G. W., Kjær, A., Lo, L.-C., Nakanishi, K., Nielsen, R. I., Olsen, C. E., Pedersen, C. and Stidsen, C. E., 1994. Malonofungin: an Antifungal Aminomalonic Acid from *Phaeoramularia fusimaculans* – Acta Chem. Scand. 48: 240–251 © Acta Chemica Scandinavica 1994.

In screening for antifungal metabolites, a novel compound, malonofungin, exhibiting growth inhibitory activity against *Botrytis cinerea* (grey mould), has been isolated from fermentations of *Phaeoramularia fusimaculans* CBS 616.87. Its structure is established as (*E*)-(3*R*,4*S*,5*S*)-5-acetoxy-2-amino-2-carboxy-3,4-dihydroxy-14-oxoicos-6-enoic acid, representing an addition to the rare class of naturally occurring aminomalonic acids. ^{1}H NMR data and extensive use of CD spectroscopy have been utilized to establish the absolute stereochemistry of malonofungin. The structural and biological relationship of malonofungin to previously reported fungal metabolites is discussed.

As part of a screening programme for microbial metabolites with growth inhibitory activity against phytopathogenic fungi, a novel compound, designated malonofungin, has been isolated from fermentations of a fungus originating from Jamaican *Panicum maximum* leaves and identified as *Phaeoramularia fusimaculans*. We report on the production, isolation, structure elucidation, including stereochemistry, and biological properties of malonofungin, which displays inhibitory action on the *in vitro* growth of *Botrytis cinerea* (grey mould). The structural and biological relationship to various, previously reported fungal metabolites is presented and discussed.

Characterization and purification

Thin layer chromatography (TLC) of the crude mycelium extract (see the Experimental), followed by bioautography with *Botrytis cinerea* as a test organism, revealed activity within a spot responding positively to spraying with 2,4-dinitrophenylhydrazine and ninhydrin, thus deriving from a metabolite that contains carbonyl and, most likely, amino acid functionalities. Agarose gel electrophoresis, monitored by bioautography, showed the activity to be associated with a negatively charged species at neutral pH. Limited stability of the active principle in aqueous solutions called for temperatures below 10 °C throughout all purification operations. Another complication arose from the fact that standard, silica-based chromatographic materials induced decomposition and loss of activity. Hence, recourse was taken to HPLC purification using a matrix based on styrene/DVB. From this material the active component could be eluted with only moderate loss of activity by an aqueous acetonitrile gradient buffered with ammonium acetate. Fractions were monitored by *in vitro* activity against *Botrytis cinerea* and freed of volatile buffer material and solvents by lyophilization. Repetition of this procedure afforded homogeneous malonofungin as a colourless, amorphous solid (see the Experimental).

Structure elucidation

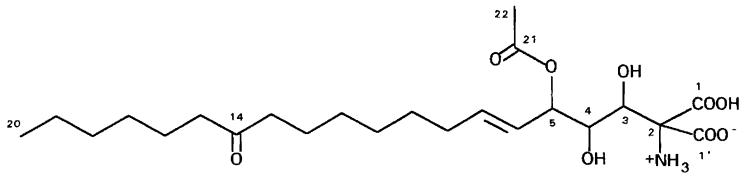
^{1}H and ^{13}C NMR spectra of malonofungin were recorded in CD_3OD at 0 °C, the optimal compromise between solubility and stability. In the ^{13}C spectrum, twenty-three lines were observed, attributable, according to multiplicity analysis (DEPT), to two CH_3O, eleven aliphatic CH_2, three aliphatic CH, two vinylic CH, one aliphatic C, and four C = O groups (one ketone, and three acids or acid derivatives). The ^{1}H NMR spectrum provided

* To whom correspondence should be sent.

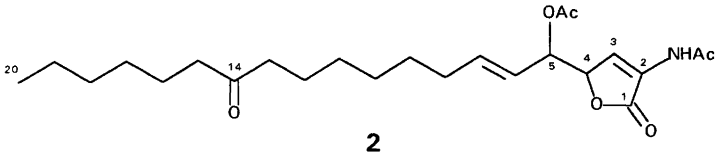
Table 1. ^{1}H and ^{13}C NMR data of malonofungin in CD_3OD at 273 K.

Position[a]	δ_C[b]	δ_H[c]	HMBC[d]
1	171.3 (s)	–	–
1′	170.6 (s)	–	–
2	75.3 (s)	–	–
3	70.1 (d)	4.76 (d;1)	C-1/C-1′,C-2,C-4
4	74.5 (d)	3.96 (dd;1,9)	C-5
5	78.5 (d)	5.38 (t;8)	C-4,C-6,C-7,C-21
6	125.7 (d)	5.44 (dd;8,15)	C-5,C-7
7	138.8 (d)	5.86 (dt;7,15)	C-5,C-6,C-8,C-9
8	33.5 (t)	2.06 (m;7)	–
9	29.8 (t)	1.40 (m)	C-10
10	30.0 (t)†	1.1–1.3 (m)	–
11	30.1 (t)†	1.1–1.3 (m)	–
12	24.8 (t)	1.52 (m)	C-11
13	43.4 (t)	2.44 (t;7)	C-11,C-12,C-14
14	214.0 (s)	–	–
15	43.5 (t)	2.44 (t;7)	C-14,C-16,C-17
16	24.8 (t)	1.52 (m)	C-17
17	30.2 (t)†	1.1–1.3 (m)	–
18	32.9 (t)	1.1–1.3 (m)	–
19	23.7 (t)	1.1–1.3 (m)	–
20	14.5 (q)	0.90 (t;7)	C-18,C-19
21	172.5 (s)	–	–
22	21.4 (q)	2.04 (s)	C-21

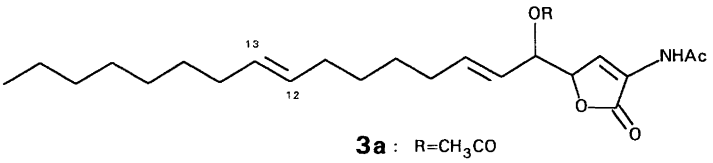
[a] Numbering according to formula **1**. [b] δ-Values relative to solvent peak at 49.0 ppm. Shifts with the same superscript are interchangeable. [c] δ-Values relative to solvent peak at 3.30 ppm; figures in parentheses are coupling constants in Hz. [d] Correlations observed in the HMBC spectrum, optimized for $J_{CH}=6$ Hz.



1



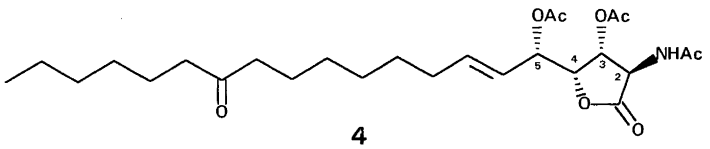
2



3a : R=CH_3CO

3b : R=H

3c : R=C_6H_5CO

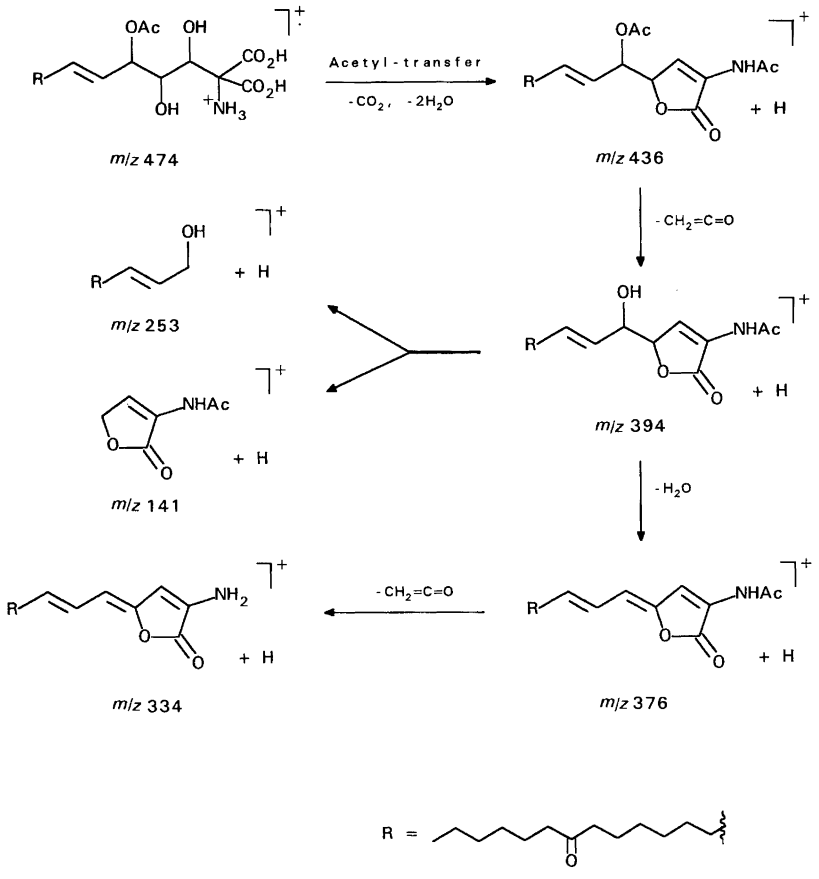


4

confirmatory evidence for one singlet and one triplet CH_3 group, a disubstituted *trans*-olefin (J = 15 Hz), three electronegatively substituted methine groups, and a linear array of a total of eleven CH_2 groups, interspaced by a carbonyl group. The location of the latter was partly defined by two four-proton signals attributable to two pairs of isochronous methylene protons positioned α and β to the carbonyl group. Of the remaining seven methylenes, six showed up in a broad, non-resolved, twelve-proton signal at δ 1.1–1.4 whereas the last group exhibited a signal at lower field (δ 2.06). The observed NMR signals are listed in Table 1.

Further information about the molecular composition and structural details was provided by mass spectroscopy. High-resolution liquid secondary ion mass spectrometry (HR-LSIMS), performed in 3-nitrobenzyl alcohol in the negative ion mode, gave rise to an intense pseudomolecular ion signal at *m/z* 472 ($C_{23}H_{39}NO_9$–H). HR-MS (CI-CH_4) afforded diagnostically useful ions at *m/z* 113 ($C_7H_{13}O$) and *m/z* 85 (C_6H_{13}), suggesting the heptanoyl grouping as a structural element of malonofungin. Initially, spectral features in the CIMS, including a C_{24}-ion, were difficult to reconcile with the established molecular weight (see below). The molecular composition, along with the NMR-established existence of thirty-three non-exchangeable protons, one alkene and four carbonyl double bonds, served to ascertain: (i) the acyclic character of malonofungin, and (ii) its content of six labile protons. The combined evidence suggested the overall structure **1** for malonofungin. The ^{1}H–^{1}H COSY spectrum, together with direct and long-range ^{1}H-detected heteronuclear shift correlation spectroscopy (HMQC and HMBC), supported the proposed structure and allowed the assignment of the ^{1}H and ^{13}C resonances presented in Table 1. Thus, the HMBC-experiment, optimized for a long-range ^{1}H–^{13}C coupling of 6 Hz, unambiguously established the position of the acetyl group on oxygen in view of an observed correlation from H-5 (δ 5.38) to C-21 (δ 172.5). Again, correlation was observed between H-3 (δ 4.79) and the quaternary C-2 (δ 75.3), thus confirming the C-2,C-3 connection. Additional correlations supporting the proposed structure and spectral assignment are given in Table 1. The aminomalonic acid character of **1** fits well with an observed *m/z* 428 fragment in its LSIMS, recorded in the negative ion mode, corresponding to the loss of the elements of CO_2 from the pseudomolecular ion.

An unexpected HR-MS (CI) pattern of malonofungin deserves comment. Intense ions were observed, in isobutane at *m/z* 436 ($C_{24}H_{37}NO_6$ + H), 394 ($C_{22}H_{35}NO_5$ + H), and 376 ($C_{22}H_{33}NO_4$ + H), in ammonia at *m/z* 334 ($C_{20}H_{31}NO_3$ + H), and in isobutane and ammonia, at *m/z*



Scheme 1.

253 ($C_{16}H_{29}O_2$), and *m/z* 142 ($C_6H_7NO_3$ + H), suggesting that fragmentation had occurred from a non-observed mother ion (*m/z* 516), conceivably produced from the non-observed molecular ion *m/z* 474 (*M* + H) by intermolecular transacetylation. Stabilization of the former ion by decarboxylation, lactonization, and dehydration would account for the *m/z* 436 ion, whence further fragmentation proceeds unexceptionally (Scheme 1). An analogous facile O→N acyl migration from an allylic acetate to an amino group in a closely related α-amino acid has recently been reported within the sphingofungin series (see the Discussion).

Various attempts at stabilizing malonofungin by chemical modifications such as catalytic or borohydride reduction and methylation, proved unsuccessful. Acetylation, however, afforded a crystalline, stable acetyl derivative (**2**) in moderate yields. Its UV absorption at 246 nm and the presence of two additional alkene carbon signals in its ^{13}C NMR spectrum (Table 2) suggested the introduction of an α,β-unsaturated carbonyl system into a molecule still containing a total of four carbonyl groups. Since the presence of two acetyl groups in the acetyl derivative was evident from its ^1H NMR spectrum (Table 2), acetylation must have been accompanied by decarboxylation. A weak molecular ion at *m/z* 435 (M^+), accompanied by an intense *m/z* ion (*M*–AcOH)($C_{22}H_{33}NO_4$), observed in the HR-MS (EI) of the acetyl derivative, was in accordance with the introduction of an additional acetyl group, accompanied by decarboxylation and loss of two molecules of water, one giving rise to the α,β-unsaturated carbonyl system, the other, by necessity, to cyclization. The NMR data, presented in Table 2, supplemented by direct and long-range ^1H–^{13}C correlations observed in HMQC and HMBC experiments, and UV and MS data, together established **2** as the overall structure of the acetyl derivative.

A second, minor product was isolated from the acetylation mixture, differing from **2** by its lack of UV absorption at about 250 nm and by containing an additional acetoxy group and two extra hydrogen atoms, as confirmed by a pseudomolecular ion at *m/z* 496 (*M* + H) in its LSIMS. Obviously, we were here dealing with a 2,3-dihydro-3-acetoxy derivative of **2** to which we shall return in the following discussion on stereochemistry.

The laborious, low-yield isolation of **1** as a source of **2** prompted attempts at acetylation of a crude metabolite fraction, followed by isolation and purification of the acetylated products; this proved to be an obvious improvement over acetylation of prepurified **1** for producing additional quantities of acetylated products. In one such acetylated fermentation batch, **2** was accompanied by a second, and major, acetate differing slightly in chromatographic behaviour. The purified congener exhibited NMR spectra (Table 2) revealing its close structural relationship to **2**. Lack of the ketone function, the appearance of a novel 1,2-disubstituted alkene, the presence in the MS

Table 2. ^1H and ^{13}C NMR data of the acetyl derivatives **2**, **3a** and **4** in $CDCl_3$.

	2		**3a**		**4**
Position[a]	δ_C[b]	δ_H[c]	δ_C[b]	δ_H[c]	δ_H[c]
1	169.1 (s)	–	169.2 (s)	–	–
2	127.0 (s)	–	127.0 (s)	–	4.56 (t;8)
3	125.3 (d)	7.35 (d;2)	125.3 (d)	7.35 (d;2)	4.55 (t;8)
4	81.4 (d)	5.09 (dd;2,5)	81.8 (d)	5.09 (dd;2,6)	4.88 (dd;2,8)
5	73.9 (d)	5.38 (m)	73.9 (d)	5.38 (m)	5.40 (dd;2,7)
6	122.3 (d)	5.33 (m)	122.3 (d)	5.39 (m)	5.50 (m)
7	139.0 (d)	5.85 (dt;7,15)	139.0 (d)	5.87 (dt;7,15)	5.87 (dt;7,16)
8	32.1 (t)	2.04 (m)	32.2 (t)	2.05 (m)	2.05 (m)
9	28.3 (t)	1.36 (m)	28.0 (t)	1.2–1.3 (m)	1.36 (m)
10	31.6 (t)[§]	1.2–1.3 (m)	29.1 (t)	1.2–1.3 (m)	1.2–1.3 (m)
11	28.7 (t)[§]	1.2–1.3 (m)	32.5 (t)	1.97 (m)	1.2–1.3 (m)
12	23.7 (t)[†]	1.56 (m)	129.8 (d)[†]	5.39 (m)	1.56 (m)
13	42.7 (t)[‡]	2.39 (m)	130.7 (d)[†]	5.39 (m)	2.40 (t)
14	212.0 (s)	–	32.1 (t)	1.97 (m)	–
15	42.9 (t)[‡]	2.39 (m)	29.0 (t)[§]	1.2–1.3(m)	2.40 (t)
16	23.8 (t)[†]	1.56 (m)	24.7 (t)[§]	1.2–1.3 (m)	1.56 (m)
17	28.9 (t)[§]	1.2–1.3 (m)	29.6 (t)[§]	1.2–1.3 (m)	1.2–1.3 (m)
18	28.9 (t)[§]	1.2–1.3 (m)	31.8 (t)	1.2–1.3 (m)	1.2–1.3 (m)
19	22.5 (t)	1.2–1.3 (m)	22.6 (t)	1.2–1.3 (m)	1.2–1.3 (m)
20	14.0 (q)	0.89 (t,7)	14.1 (q)	0.88 (t;7)	0.89 (t;7)
OAc	21.0 (q)	2.07 (s)	21.0 (q)	2.08 (s)	2.12/2.13 (s)
	169.6 (s)	–	169.5 (s)	–	–
NAc	23.6 (q)	2.20 (s)	23.6 (q)	2.20 (s)	2.09 (s)
	169.0 (s)	–	168.6 (s)	–	–
NH	–	7.85 (br s)	–	7.91 (br s)	6.05 (d;8)

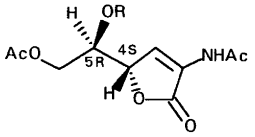
[a]Numbering according to formulae **2**, **3a** and **4**. [b]δ-Values relative to solvent peak at 77.0 ppm. Shifts with the same superscripts are interchangeable. [c]δ-Values relative to solvent peak at 7.27 ppm; figures in parentheses are coupling constants in Hz.

(CI) of a pseudomolecular ion at m/z 420 ($M+H$), together with observed direct and long-range $^{1}H-^{13}C$ correlations in the HMQC and HMBC spectra all fit well with a structure differing from **2** by possessing a double bond instead of the keto group in its chain. In order to locate the former, the lactone was subjected to epoxidation, yielding a monoepoxide that, according to NMR analysis, was still both an allylic acetate and unsaturated lactone. Treatment of the epoxide with periodic acid afforded octanal, identified by GLC–MS, a fact placing the double bond at the C12–C13 position. Increased intensity of the 965 cm^{-1} *trans*-olefin band, compared with that of **2**, along with lack of absorption within the 675–730 cm^{-1} range, characteristic of 1,2-disubstituted *Z*-olefins, in the IR spectrum, served to establish the *E*-configuration of the alkene grouping and hence the structure **3a** for the lactone congener. In view of the derivation of **2** from **1** upon acetylation, it is quite feasible to suggest a similar derivation of **3a** from an unobserved metabolic precursor identical with **1** save for the chain modification.

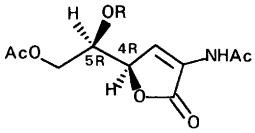
Stereochemistry

The three stereogenic centres in **1** (C-3, C-4, and C-5) are retained in the 2,3-dihydro-3-acetoxy derivative, the minor product from the acetylation of **1**. Only one diastereomer was encountered suggesting a highly stereoselective course of the decarboxylation step. NOE difference experiments proved useful in establishing the relative configurations at C-2, C-3, and C-4 of the acetylation product. Thus, irradiation of H-3 caused a significant enhancement of the H-4 signal (10%) and *vice versa* (8%), whereas no responses were noted between H-2 and H-3. These results render structures with a *syn*-relationship of the methine hydrogens at C-3 and C-4, such as **4**, likely for the minor acetylation product. In view of the lack of conformational information about the exocyclic substituent of **4**, an observed NOE (4%) of H-5 on irradiation of H-4 carries no useful information regarding the configuration at C-5. Recourse was therefore taken to CD spectroscopy in order to establish the absolute configurations at C-4 and C-5 of **2** and **3a** and hence of malonofungin (**1**) and its likely, though non-observed 14-deoxo-12,13-didehydro congener.

Absolute stereochemistry at C-4. The absolute stereochemistry of endo-unsaturated lactones has been determined from the sign of the Cotton effects of their CD spectra in the region of 220–260 nm.[1,2] Authentic specimens of the acetylated enaminelactones (**5a**)[3,4] (leptosphaerin diacetate[5]), and **6a**, previously unknown, were produced from *N*-acetyl-D-mannosamine and *N*-acetyl-D-galactosamine, respectively, by oxidation with aqueous bromine, followed by acetylation according to the general method of Pravdić and Fletcher.[3] The CD spectra of compounds **5a** and **6a**, differing solely in the configurations at C-4, show



5a: R=CH_3CO
5b: R=H
5c: R=C_6H_5CO



6a: R=CH_3CO
6b: R=H
6c: R=C_6H_5CO

nearly the same magnitude but are of opposite sign in the range 220–260 nm (Fig. 1) (Table 3) as in a pair of mirror images. Since **5a** and **6a** are a diastereomeric pair with different configurations at C-4, i.e. *S* in **5a**, *R* in **6a**, the enantiomeric relationship of the CD curves shows that the signs are largely dependent on the chirality at C-4, while receiving negligible effect from the 5-OAc. Based on these results we can conclude that a positive or negative band at ca. 240 nm corresponds to a 4*S* or 4*R* configuration, respectively. The CD spectra of compounds **3a** and **2** show a positive band in the range 220–260 nm (Fig. 2) (Table 3), thus demonstrating that they both have a 4*S*-configuration.

Absolute stereochemistry at C-5. The CD spectra of allylic benzoates have been extensively used to determine the absolute stereochemistry of various compounds.[6–9] Since compounds **3a** and **2** both have an allylic system, we envisioned that their benzoate derivatives would provide information about the absolute stereochemistry at C-5. Moreover, interaction between the ene–lactone and chromophores such as a benzoate at C-5 could also be utilized to unveil the absolute stereochemistry at C-5. Two model benzoate derivatives, **5c** and **6c**, were prepared from compounds **5a** and **6a** to evaluate the interaction between the 5-benzoate and the ene–lactone system. Compounds **5a** and **6a** were subjected to various basic conditions, in an attempt selectively to remove the 5-acetyl group. However, several side reactions,[4] such as

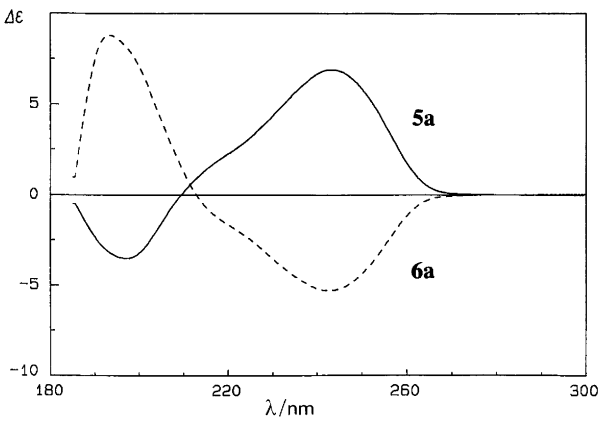


Fig. 1. CD curves of compounds **5a** and **6a**.

Table 3. CD data, in TFE and at room temperature, for the various compounds studied (wavelenghts in nm, Δε in parentheses).

Compound	CD
3a	209 (−3.4), 245 (+5.9)
2	209 (−3.5), 246 (+5.8)
5a	197 (−3.5), 218sh (+1.8), 243 (+6.9)
6a	194 (+8.8), 219sh (−1.4), 243 (−5.3)
5c	204 (−3.2), 229 (+7.6), 247 (+0.5), 255 (+1.2)
6c	209 (+0.9), 225 (+5.7), 245 (−18.0)
3c	199 (−12.3), 211 (−2.3), 226 (−6.2), 246 (+10.0)

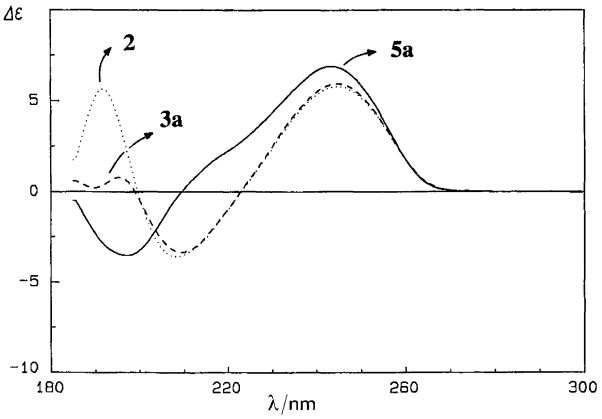
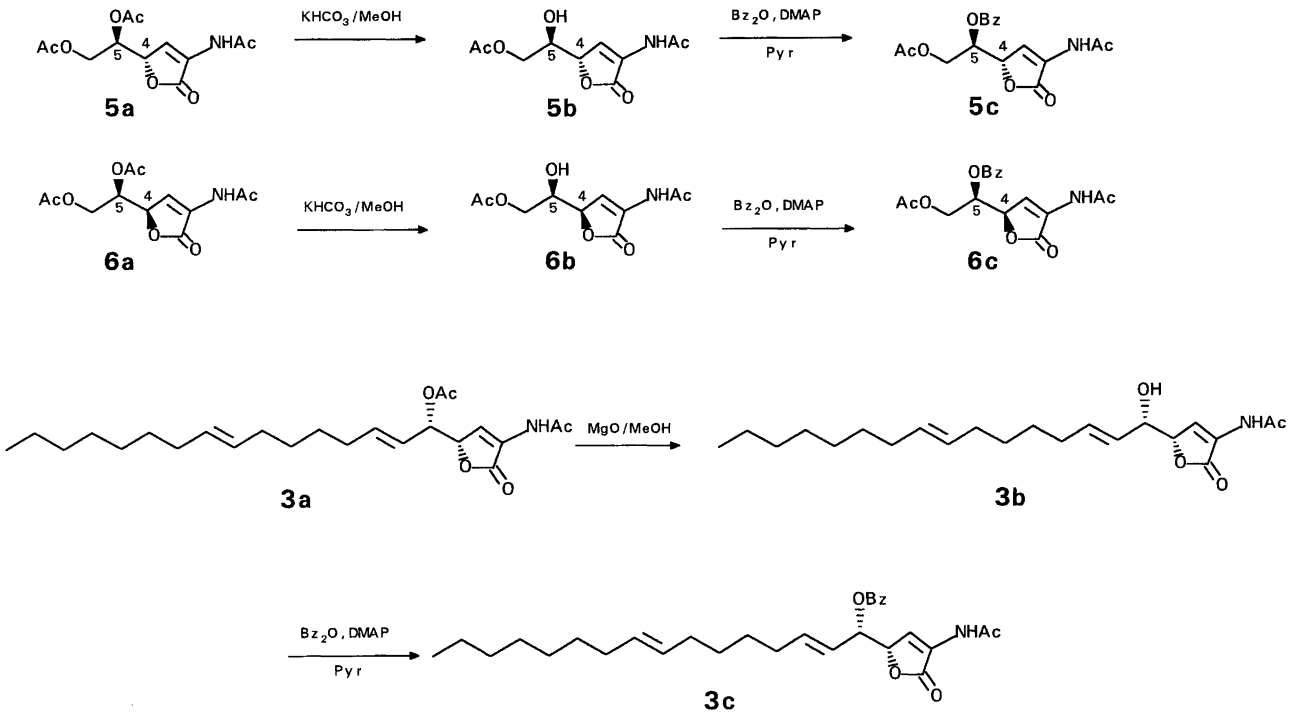


Fig. 2. CD curves of compounds **2**, **3a** and **5a**.

elimination to form the diene–lactone, de-*N*-acetylation, and opening of the lactone ring, occurred besides the desired reaction under these conditions. After several attempts, it was found that deacetylation could be achieved with $KHCO_3$ in methanol to yield the desired products **5b** and **6b**, albeit in low yields. MgO in methanol[10] gave similar results but at a slower rate. Compounds **5b** and **6b** were then benzoylated with Bz_2O–DMAP in pyridine to offer the benzoate derivatives **5c** and **6c** (Scheme 2). Similarly, benzoate **3c** was prepared from compound **3a** by the same method. Selective C-5 deacetylation and benzoylation in compounds **5a**, **6a** and **3a** were monitored by changes of 5-H chemical shifts in their ^1H NMR spectra, i.e. 5.15 in **5a**→3.96 in **5b**→5.44 ppm in **5c**; 5.23 in **6a**→4.01 in **6b**→5.55 ppm in **6c**; and 5.34 in **3a**→4.10 in **3b**→5.62 ppm in **3c**.

Since the additivity principle[11] holds when several interacting and/or non-interacting chromophores are present in the same molecule, the CD curves of compounds **5c** and **6c** can be considered as consisting of two major contributions, namely, the isolated ene–lactone and the coupled 5-benzoate/ene–lactone. The CD spectra of compounds **5a** and **6a** represent those of the isolated ene–lactones. Thus, subtraction of the CD contributions of isolated ene–lactones **5a** and **6a** from the CDs of **5c** and **6c** should disclose the coupling between the 5-benzoate and the ene–lactone. The CD curves of **5a**/**6a**,**5c**/**6c** (Table 3) and the difference spectra **5c**–**5a**/**6c**–**6a** are shown in Figs. 3 and 4. Both difference spectra



Scheme 2.

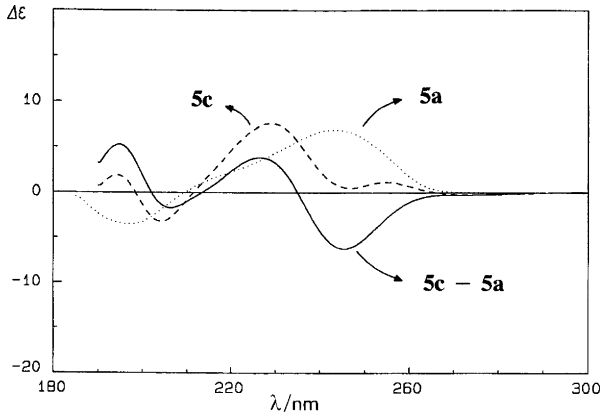


Fig. 3. CD curves of compounds **5a**, **5c**, and the difference (**5c–5a**).

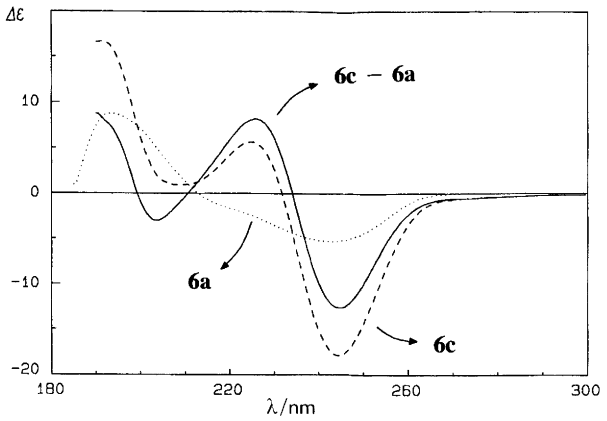
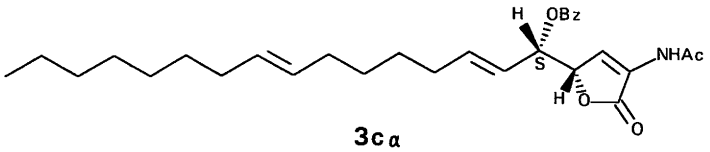


Fig. 4. CD curves of compounds **6a**, **6c**, and the difference (**6c–6a**).

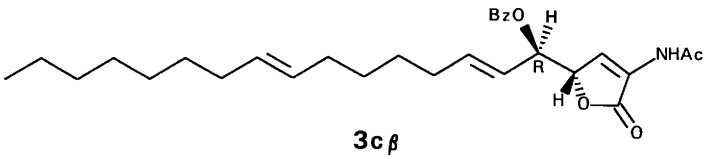
show a negative coupling (but of different magnitude) between the 5-benzoate and ene–lactone. For compound **5c** this couplet is centred at 235 nm (227 nm, Δε +3.8; 246 nm, Δε −6.2; *A* value −10), while for compound **6c** this couplet is centred at 234 nm (226 nm, Δε 8.2; 246 nm, Δε −12.7; *A* value −21). These couplets arising from the coupling between the 5-benzoate and the ene–lactone are both seen to be negative, independent of the C-4 configuration, that is, although the split CD curves differ in amplitudes, they are both negative when the benzoate group at C-5 is *R*.

With the coupling between 5-benzoate and the ene–lactone clarified, we were then able to use this interaction to decipher the absolute configuration of compound **3c** at C-5. The CD of benzoate **3c** consists of three major contributions: (a) allylic benzoate coupling, (b) 5-benzoate/ene–lactone coupling, and (c) isolated ene–lactone. Of these three, (a) and (b) can be used independently to establish the stereochemistry at C-5. Since the chiral centre at C-4 has already been assigned 4*S*, only two possibilities are left for compound **3c**, either **3cα** or **3cβ**.

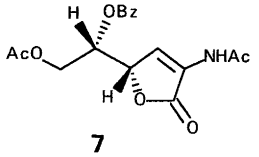
In order to obtain the allylic benzoate contribution (a) in compound **3c**, difference CD operations were performed with compound **3c** to remove the contributions of (b) and (c). This technique has been employed before[8,9] to reveal the signs of net allylic benzoate interactions. If **3cα** is the correct structure, compound **7** should be used as the model to be subtracted, whereas if **3cβ** is the correct structure, then compound **5c** should be used. Since the correct structure was not known at this stage, the difference operations with both models, **7** and **5c**, were carried out to obtain two CD curves (**3c** − **7** and **3c** − **5c**); curve (**3c** − **7**) shows a single negative band at 242 nm (Δε −8.4) (Fig. 5) while curve (**3c** − **5c**) shows a positively split CD centred at 238 nm (227 nm, Δε −13.4; 246 nm, Δε +9.4; *A* value +23) (Fig. 6). Even though compound **7** is not available, its CD can be generated from that of **6c**, its enantiomer.



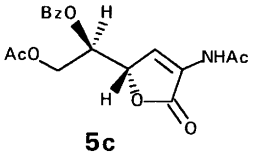
3cα



3cβ



7



5c

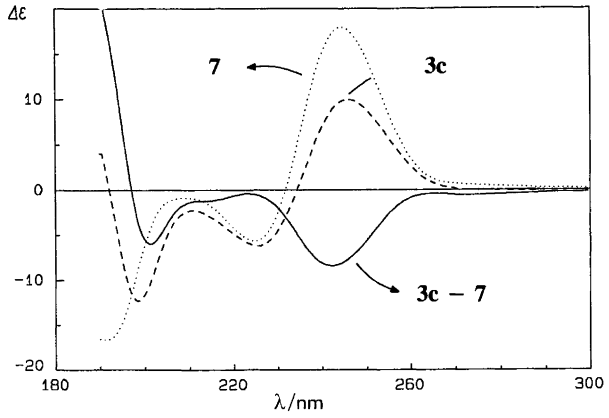


Fig. 5. CD curves of compounds **3c**, **7**, and the difference (**3c**−**7**).

Here we face two possible explanations for these two CD curves. If **3cα** is the correct structure, curve (**3c** – **7**) should represent the curve for allylic benzoate, and curve (**3c** – **5c**) should represent the sum of allylic benzoate and benzoate/ene–lactone. On the other hand, if **3cβ** is the correct structure, curve (**3c** – **7**) will be the sum of allylic benzoate and benzoate/ene–lactone, and curve (**3c** – **5c**) will be the curve for allylic benzoate. Based on the fact that allylic benzoates show a single band in the region 230–240 nm[6–9] (in the present case, negative for 5*S* and positive for 5*R*), whereas benzoate/ene–lactones show split couplets (positive for 5*S* and negative for 5*R*), we found that only the former **3cα** meets both criteria. The absolute stereochemistry at C-5 of compound **3c** is then 5*S*.

From the results of CD spectroscopic studies on two series of model compounds, the absolute stereochemistry at C-4 and C-5 of compounds **3a** and **2** have been determined to be 4*S* and 5*S*. Consequently, the saturated lactone produced from malonofungin upon acetylation, is represented by the absolute stereochemistry depicted in **4**. Since formation of the latter does not involve C-3, C-4, and C-5, the structure of malonofungin as (*E*)-(3*R*,4*S*,5*S*)-5-acetoxy-2-amino-2-carboxy-3,4-dihydroxy-14-oxoicos-6-enoic acid (**8**) has been established.

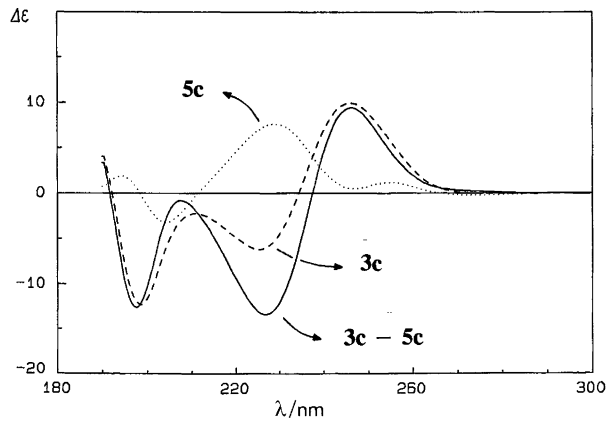


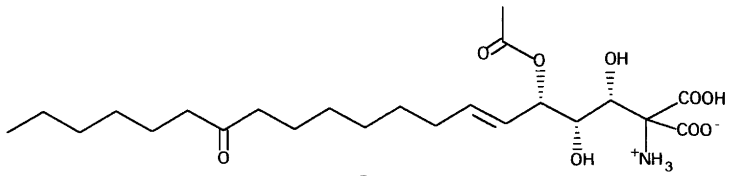
Fig. 6. CD curves of compounds **3c**, **5c**, and the difference (**3c**−**5c**).

Discussion

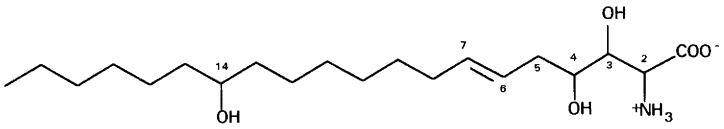
Structurally, malonofungin (**8**) can be construed as a derivative of an (*E*)-2-amino-3,4,14-trihydroxyicos-6-enoic acid (**9**), modified by carboxy-substitution at C-2 and oxidation at C-14 and C-5 to a keto and allylic hydroxy group, respectively, the latter equipped with an acetyl group. In that light, malonofungin constitutes a novel member of a long known family of antifungal metabolites, comprising myriocin (identical with thermozymocidin) (**10**)[12] and fumifungin (**11**),[13] recently supplemented by a group of six antifungal agents, named sphingofungins A–D (**12–15**)[14] and sphingofungins E and F (**16**, **17**),[15] isolated from strains of *Aspergillus fumigatus* and *Paecilomyces variotii*, respectively. The reported facile O→N acetyl migration converting **14** into **15**,[14b] is of interest in connection with the mass spectrometric behaviour of malonofungin discussed above. That the similarity of malonofungin (**8**), myriocin (**10**), and the extensive C_{20}-series cuts deep is reflected in the identical absolute configurations at C-3, C-4, and, where relevant, C-5, in **8**, **10**, and **12–15**, suggesting closely related biosynthetic pathways. Inasmuch as thermozymocidin (myriocin) (**10**) has been shown to derive from L-serine and stearoyl-SCoA[16] it is reasonable to envision the aminomalonic acid moiety in malonofungin (**8**) as arising from oxidation of a primary hydroxy group such as that in **10**. The biosynthetic origin of the C_{20}-compounds **11–15** is unknown. Stereospecific involvement of glycine or decarboxylation of serine-derived aminomalonic acid intermediates are possibilities to be considered along with the decarboxylative serine pathway firmly established in the biosynthesis of sphingosines.

Malonofungin exhibits a biological activity spectrum comparable to those reported for the structurally related compounds described above. Thus, it shows broad growth inhibitory effects against a range of fungi belonging to the genera *Botrytis*, *Pyricularia*, *Fusarium* and *Penicillium* but not against bacteria or yeast. No antifungal activity was observed for the acetylated compounds **2** and **3a**.

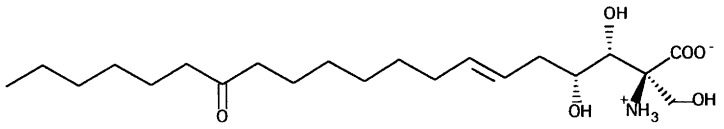
Aminomalonic acids, prone to undergo decarboxylation, are rare as natural products. A literature search revealed the antibiotic malonomicin (**18**),[17] produced by *Streptomyces rimosus*, and the fish attractant arcamine (**19**),[18] a product of a marine mussel, as the sole representatives of its class prior to the identification of malonofungin (**8**). However, the labile character of the aminomalonic acid moiety may well have allowed additional members of this class to pass unnoticed as natural products.



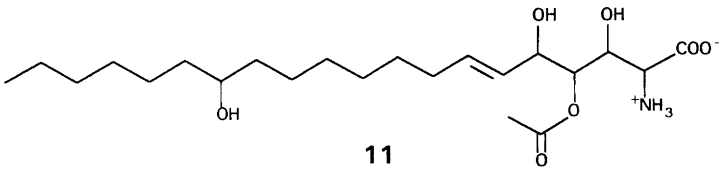
8



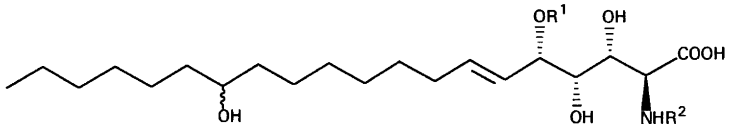
9



10



11

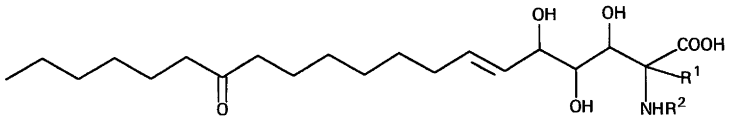


12 : SphA: R^1=H; R^2=C(NH)NH_2

13 : SphB: R^1=R^2=H

14 : SphC: R^1=OAc; R^2=H

15 : SphD: R^1=H; R^2=Ac

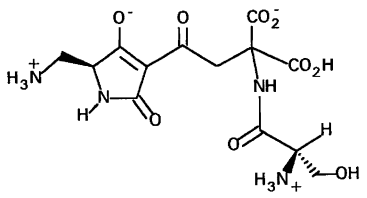


16: SphE: R^1=CH_2OH

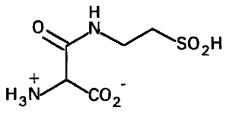
17: SphF: R^1=CH_3

Experimental

Melting points are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. CD spectra were recorded on a JASCO J-720 spectropolarimeter in trifluoroethanol (TFE) and processed with software developed in the American laboratory. UV spectra were obtained on a Perkin-Elmer Lambda 4B UV–VIS spectrophotometer. IR-spectra were recorded (KBr) on a Perkin-Elmer 1720 instrument, the ^1H and ^{13}C NMR spectra on Bruker AM500 and AC300P instruments, or on a Varian VXR-400, 300, or 200 MHz spectrometer as noted. The ^1H-detected ^1H–^{13}C-correlation experiments (HMQC and HMBC) were performed on the AC300P instrument equipped with a conventional ^1H–^{13}C dual probe. High resolution mass spectra were recorded at 70 eV ionization potential on a VG70-250SQ or a VG70-250SE instrument; methane, isobutane and ammonia were used for chemical ionization (CI); HR-LSI mass spectra were obtained in 3-nitrobenzyl alcohol on the latter instrument, using cesium ion bombardment at 12 kV and detection of positive and negative ions; the EI-spectra are presented as *m/z* (% rel. int.). For HPLC purifications, two Waters 6000 pumps, controlled by Waters Maxima 820 software, and UV detection by means of a Hewlett-Packard 1040 diode array detector, were employed. All solvents used



18



19

were of HPLC grade. Pyridine was dried by distillation over CaO before use.

Fermentation and extraction. The fungus, identified as *Phaeoramularia fusimaculans* and deposited at the *Centraalbureau voor Schimmelculturen (CBS)*, Holland, with the designation CBS 616.87, was fermented either in surface cultures or submerged in shake flasks on a yeast extract–glucose medium. The fungus was grown at 20–25 °C for 7 days or longer on slants containing 12 ml of YPG agar. The YPG agar was prepared by dissolving yeast extract (4.0 g), KH_2PO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), glucose (15 g), and Bacto™ (Difco) (20 g) in demineralized water (1 l). The substrate was autoclaved at 121 °C for 20 min. The substrate for shake-flask fermentation was prepared by dissolving yeast extract (4.0 g), KH_2PO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), glucose (15 g), and Pluronic™L61 (BASF) (0.1 g) in demineralized water (1 l). The substrate was autoclaved at 121 °C for 20 min. Erlenmeyer flasks (500 ml), each containing 100 ml of substrate, were inoculated with 10^6 spores of *Phaeoramularia fusimaculans* CBS 616.87 from a YPG agar slant. The flasks were shaken (230 rpm) at 25 °C for 3–7 days. The mycelium was separated by centrifugation and extracted with EtOH (100 ml per flask), keeping the temperature below 10 °C to minimize loss of activity. The extract was diluted with three volumes of water, Amberlite XAD-8 (Rhom and Haas) (10 g l^{-1}) was added, followed by stirring overnight at 5 °C. The resin was filtered off, washed with four volumes of 0.025 M phosphate buffer (pH 6.0), and the active compound eluted three times, each with three resin volumes of 60% EtOH. The combined EtOH eluates were adjusted with water to an EtOH concentration of 50% w/w ($d = 0.922$ g ml^{-1}) and decolourized by stirring with charcoal (Norit CG1) (about 1%) for 2 h. After filtration, the extract was concentrated under reduced pressure at a temperature not exceeding 5 °C until a precipitate had formed. The solid, containing the desired metabolite, was removed by centrifugation and the procedure repeated until no more solid was formed. The combined precipitates were dissolved in 50% EtOH for further HPLC purification. A minor, additional amount of the active component was recovered from the original centrifugate.

Bioassay. Spores of *Botrytis cinerea* (Novo Nordisk A/S Strain Collection) (about 10^6) were added to a salt solution (50 ml), prepared by dissolving $(NH_4)_2HPO_4$ (66 mg), KH_2PO_4 (68 mg), K_2HPO_4 (87 mg), $CaCl_2,2H_2O$ (7.4 mg) and $MgCl_2,6H_2O$ (10 mg) in distilled water (1 l), followed by sterilization at 121 °C for 20 min. The spore suspension was mixed with YPG agar (50 ml) at 30–45 °C. Portions hereof (12 ml) were poured into 9 cm Petri dishes and allowed to solidify. Wells (6 mm) were punched into the agar and 15 µl of the sample to be tested applied. After incubation at 20–25 °C for 2 days, inhibition zones around the wells were measured.

Purification and properties. Thin layer chromatography (TLC), serving to trace the active component, was performed on cellulose plates (Merck Cellulose F, 20 x 20 cm, 0.1 mm), with the upper layer of a butanol–ethanol–water 4:1:3 (v/v/v) mixture as the mobile phase. The active component, malonofungin, was visualized by spraying with ninhydrin, potassium permanganate, or 2,4-dinitrophenylhydrazine.

Purification of the crude metabolite fraction was performed by HPLC, with a PLRP-S reversed-phase material (20 µm, 100 Å) of the styrene/DVB type (Polymer Labs., UK) serving as the solid support (16 x 250 mm steel column). Gradient elution was performed, involving two eluents: (A) a 1 M ammonium acetate buffer (pH 6.0), and (B) acetonitrile, applied with a flow rate of 5 ml min^{-1} and a gradient profile starting at 27% B and rising to 70% B over 50 min. Fractions (5 ml) were collected and assayed for activity against *Botrytis cinerea*. The active fractions were combined, concentrated *in vacuo* at 5 °C, and rechromatographed in the same system until a homogeneous specimen of malonofungin resulted as an amorphous, colourless solid. Though apparently infinitely stable in the solid state when stored at − 20 °C, malonofungin rapidly undergoes decomposition, with concomitant loss of biological activity, in aqueous solutions at ambient temperature or lower.

Malonofungin (**8**) exhibits no distinct absorption in the UV region above 200 nm. Its IR spectrum displays strong signals at 3200br (NH, OH, assoc.), 2940, 2860 (CH_2), 1760 (C = O), 1720 (C = O), 1650 (C = C), 1100 (C–O), and 968 (HC = CH) cm^{-1}. The assigned ^1H and ^{13}C NMR spectra of malonofungin (**8**) are presented in Table 1. HR-LSIMS (negative mode), exhibited a strong pseudomolecular ion (*M*–H): found 472.2592, calc. for $C_{23}H_{38}NO_9$ 472.2547, and an intense [*M*–CO_2–H] ion: found 428.2665, calc. for $C_{22}H_{38}NO_7$ 428.2648.

Acetylation of malonofungin and its congener. To an ice-cooled solution of malonofungin (**8**) (6.5 mg) in pyridine

(1.0 ml), acetic anhydride (0.5 ml) was added dropwise with stirring and cooling. The mixture was kept overnight at 4 °C. The solution was stirred with ice (5 g) for 15 min, and the mixture extracted with three 5 ml portions of chloroform. The organic phase was washed with H_2SO_4 (2 M), water, satd. $NaHCO_3$ solution, and water, then dried (Na_2SO_4) and evaporated *in vacuo* to yield a yellow oil (9.0 mg). This was purified by reversed-phase HPLC (LiChroSorb RP18, 250 × 10 mm, 7 μm), applying a linear gradient from 55% to 73% acetonitrile over 50 min, a flow rate of 6.5 ml min^{-1} and UV detection (215 nm), to yield the homogeneous acetyl derivative **2** (1.9 mg), which slowly crystallized from EtOAc–heptane. M.p. 66 °C. HR-MS (EI): *m/z* 435 (M^+); *m/z* 375 (*M*–AcOH): found 375.2406, calc. for $C_{22}H_{33}NO_4$ 375.2409. ^1H and ^{13}C NMR spectra are presented in Table 2. IR: 3310, 2920, 1782, 1771, 1734, 1707, 1650, 1532, 1375 and 1236 cm^{-1}. UV (methanol) (log ε): 246 nm (3.91). A minor, more polar component, lacking UV absorption at 246 nm, was isolated from the acetylation mixture, and assigned the structure **4** (0.5 mg). Its ^1H NMR signals are presented in Table 2. LSIMS: *m/z* 496 (*M* + H). Alternatively, the XAD-8 extract (lyophilized solid) from 15 l of culture broth was acetylated in the same fashion [acetic anhydride (12.5 ml) in pyridine (25 ml)] to give, after work-up and HPLC purification, a specimen of **2** (25 mg) with identical properties, $[\alpha]_D^{20}$ +37° (*c* 0.6, chloroform).

In one fermentation batch, the major acetylation product exhibited slightly different chromatographic behaviour and was obtained as a colourless foam, $[\alpha]_D^{20}$ +34° (*c* 0.5, chloroform). MS (CI): *m/z* 420 (*M* + H). ^1H and ^{13}C NMR data are presented in Table 2. IR: 3340, 2920, 2850, 1760, 1745, 1708, 1650, 1534, 1370, 1230, 1125, and 965 cm^{-1}. UV (methanol) (log ε): 246 nm (3.95). Monoepoxidation of the acetylation product was achieved by adding *m*-chloroperbenzoic acid (2.5 mg) to a solution of the product (3.0 mg) in dichloromethane (1 ml), supplemented, after 20 min, with another portion (1 mg) of the peracid. After 1 h at 20 °C, satur. $NaHCO_3$ solution (1 ml) was added to the mixture, and the dried organic phase was concentrated and purified by reversed-phase HPLC as described above for **2** to give a nearly 1:1 mixture of diastereomeric monoepoxides (2.0 mg). ^1H NMR ($CDCl_3$): δ 0.90 (3 H, t, *J* 7 Hz, CH_3-20), 1.2–1.5 (14 H, m), 1.52 (4 H, m, CH_2-11, CH_2-14), 2.08 (3 H, s, CH_3CO–O), 2.09 (2 H, m, CH_2-8), 2.20 (3 H, s, CH_3CO–N), 2.68 (2 H, m, CH-12, CH-13), 5.10 (1 H, dd, *J* 2 and 5 Hz, CH-4), 5.38 (1 H, m, CH-6), 5.39 (1 H, m, CH-5), 5.86 (1 H, m, CH-7), 7.35 (1 H, d, *J* 2 Hz, CH-3) and 7.63 and 7.69 (1 H, br s, NH). ^{13}C NMR ($CDCl_3$, double signals arise from the ca. 1:1 mixture of diastereomers): δ 169.5, 169.32, 169.1/169.0, 168.8/168.7, 138.6/138.5 (C-7), 127.0/126.9 (C-2), 125.1 (C-3), 122.5 (C-6), 81.4/81.3 (C-4), 73.8 (C-5), 58.93/58.89 (C-12 or C-13), 58.8/58.7 (C-13 or C-12), 32.15/32.13, 32.10, 31.9, 31.8, 29.4, 29.2, 28.35/28.34, 26.0, 25.5, 23.7 (CH_3CON), 22.6, 21.0 (CH_3COO) and 14.1 (C-20). A suspension of anhydrous periodic acid (3 mg) in dichloromethane (100 μl), containing the monoepoxide (0.5 mg) was stirred intermittently for 80 min at 20 °C. GLC–MS analysis of the solution revealed it to contain a major constituent, identified as octanal upon comparison with an authentic specimen. The above data, supplemented with IR evidence, establish **3a** as the structure of the acetylation product.

*2-Acetamido-5,6-di-*O*-acetyl-2,3-didehydro-2,3-dideoxy-*D*-erythro-hexono-1,4-lactone (***5a***).* 2-Acetamido-2-deoxy-D-mannono-1,4-lactone (100 mg), prepared by bromine oxidation of 2-acetamido-2-deoxy-D-mannose,[3] was acetylated as described above for **1**. The product was purified by preparative TLC using hexane–EtOAc 2:1 as the mobile phase. The purified product (**5a**) (52 mg) possessed physical and spectroscopic properties in agreement with those reported.[3–5] MS (EI): 286 (<0.1, *M* + 1), 285 (<0.1, *M*), 225 (7, *M*–AcOH), 183 (23), 141 (31), 123 (25) and 43 (100).

*2-Acetamido-5,6-di-*O*-acetyl-2,3-didehydro-2,3-dideoxy-*D*-threo-hexono-1,4-lactone (***6a***).* 2-Acetamido-2-deoxy-D-galactono-1,4-lactone (85 mg), prepared by bromine oxidation of 2-acetamido-2-deoxy-D-galactose,[3] was acetylated as described above for **1**. After chromatographic purification the product was recrystallized from EtOAc–hexane to yield a pure specimen of **6a** (40 mg) as colourless crystals, m.p. 109 °C. $[\alpha]_D^{20}$ +6° (*c* 1.8, chloroform). Anal. $C_{12}H_{15}NO_7$: C,H,N. ^1H NMR ($CDCl_3$): δ 2.06 (3 H, s), 2.08 (3 H, s), 2.20 (3 H, s), 4.20 (1 H, dd, *J* 12 and 6 Hz), 4.38 (1 H, dd, *J* 12 and 4 Hz), 5.25 (1 H, m), 5.27 (1 H, m), 7.37 (1 H, d, *J* 1.8 Hz) and 7.81 (1 H, br s). ^{13}C NMR ($CDCl_3$): δ 170.3, 169.6, 168.9, 168.6 (4 × C = O), 126.8 (C-2), 124.2 (C-3), 79.7 (C-4), 69.9 (C-5), 62.1 (C-6), 23.7 (CH_3CO–N), and 20.7 (2 × CH_3CO–O). IR: 3310, 1780, 1732, 1702, 1658, 1540, 1370, 1245, 1228, 1120, and 1062 cm^{-1}. UV (methanol) (log ε): 246 nm (3.95). MS (EI): 286 (<0.1, *M* + 1), 242 (<0.1, *M*–Ac), 225 (5, *M*–AcOH), 183 (20), 141 (31), 123 (28) and 43 (100).

*2-Acetamido-6-*O*-acetyl-2,3-dihydro-2,3-dideoxy-*D*-erythro-hexono-1,4-lactone (***5b***).* The lactone diacetate **5a** (4.0 mg) was dissolved in MeOH (500 μl); $KHCO_3$ (20 mg) was then added. The reaction mixture was stirred at room temperature and monitored by TLC. After about 6 h, a new spot with R_f 0.40 ($CHCl_3$–MeOH 9:1) was separated and characterized as compound **5b**. ^1H NMR (400 MHz, $CDCl_3$): δ 2.12 (3 H, s, Ac), 2.17 (3 H, s, Ac), 3.96 (1 H, m, 5-H), 4.20 (1 H, dd, *J* 12.0 and 5.8 Hz, 6-H), 4.34 (1 H, dd, *J* 12 and 3.7 Hz, 6′-H), 5.06 (1 H, dd, *J* 6.0 and 2.0 Hz, 4-H), 7.49 (1 H, s, NH) and 7.52 (1 H, d, *J* 1.9 Hz, 3-H).

*2-Acetamido-6-*O*-acetyl-5-*O*-benzoyl-2,3-didehydro-2,3-dideoxy-*D*-erythro-hexono-1,4-lactone (***5c***).* The isolated monoacetate **5b** was stirred with benzoic anhydride

(10 mg) and DMAP (2 mg) in pyridine (250 µl) for 12 h at room temperature and then concentrated to dryness. The benzoate was purified by preparative silica gel TLC (500 µl, $CHCl_3$–MeOH 60:1) to give the benzoate **5c** (0.4 mg); R_f 0.53 ($CHCl_3$–MeOH 9:1). ^1H NMR (200 MHz, $CDCl_3$): δ 2.05 (3 H, s, Ac), 2.18 (3 H, s, Ac), 4.29 (1 H, dd, *J* 12.1 and 5.4 Hz, 6-H), 4.48 (1 H, dd, *J* 12.1 and 3.8 Hz, 6′-H), 5.37 (1 H, dd, *J* 5.3 and 1.9 Hz, 4-H), 5.44 (1 H, m, 5-H), 7.40–7.62 (5 H, m, NH + 3-H + 3 Ar-H) and 8.00 (2 H, dd, *J* 8.4 and 1.4 Hz, Ar-H).

*2-Acetamido-6-*O*-acetyl-2,3-didehydro-2,3-dideoxy-*D*-*threo*-hexono-1,4-lactone (***6b***).* The lactone diacetate **6a** was similarly converted into the monoacetate **6b**. ^1H NMR (300 MHz, $CDCl_3$): δ 2.11 (3 H, s, Ac), 2.17 (3 H, s, Ac), 2.39 (1 H, d, *J* 5.3 Hz, OH), 4.01 (1 H, m, 5-H), 4.25 (2 H, dd, *J* 6.0 and 1.9 Hz, 6-H + 6′-H), 5.13 (1 H, dd, *J* 4.3 and 2.0 Hz, 4-H), 7.39 (1 H, d, *J* 2.0 Hz, 3-H) and 7.51 (1 H, s, NH).

*2-Acetamido-6-*O*-acetyl-5-*O*-benzoyl-2,3-didehydro-2,3-dideoxy-*D*-*threo*-hexono-1,4-lactone (***6c***).* Benzoylation of **6b**, as described above, afforded the benzoate (6c). ^1H NMR (400 MHz, $CDCl_3$): δ 2.06 (3 H, s, Ac), 2.11 (3 H, s, Ac), 4.37 (1 H, dd, *J* 12.0 and 6.4 Hz, 6-H), 4.46 (1 H, dd, *J* 12.0 and 4.7 Hz, 6′-H), 5.36 (1 H, dd, *J* 3.8 and 2.0 Hz, 4-H), 5.55 (1 H, m, 5-H), 7.38–7.45 (4 H, m, NH + 3-H + 2 Ar-H), 7.57 (1 H, t, *J* 7.4 Hz, Ar-H) and 7.96 (2 H, d, *J* 7.2 Hz, Ar-H).

Compound **3b**. The lactone **3a** (4.0 mg), dissolved in MeOH (1 ml), was stirred with MgO powder (10 mg) at room temperature, and the reaction was monitored by TLC. After about 25 h, a new spot with R_f 0.55 ($CHCl_3$–MeOH 9:1) was isolated and characterized as the hydroxy lactone **3b** (0.4 mg). ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (3 H, t, *J* 6.0 Hz, CH_3), 1.18–1.39 (14 H, m), 1.95 (4 H, m), 2.04 (2 H, m), 2.16 (3 H, s, Ac), 4.10 (1 H, m, 5-H), 4.95 (1 H, dd, *J* 6.2 and 2.0 Hz, 4-H), 5.36 (2 H, q, *J* 2.7 Hz, alkenic), 5.48 (1 H, dd, *J* 15.4 and 7.2 Hz, alkenic), 5.84 (1 H, m, alkenic), 7.33 (1 H, d, *J* 2.0 Hz, 3-H) and 7.47 (1 H, s, NH).

Compound **3c**. Compound **3b** was then subjected to the same benzoylation conditions as described above for compound **5b** to give the benzoate **3c** (0.6 mg). R_f 0.53 (hexane–EtOAc, 1:1). ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (3 H, t, *J* 7.1 Hz, CH_3), 1.17–1.38 (14 H, m), 1.92 (4 H, m), 2.04 (2 H, q, *J* 6.9 Hz), 2.15 (3 H, s, Ac), 5.22 (1 H, dd, *J* 5.4 and 2.0 Hz), 5.34 (2 H, q, *J* 4.7 Hz, alkenic), 5.49 (1 H, dd, *J* 15.4 and 7.7 Hz, alkenic), 5.62 (1 H, dd, *J* 7.8 and 5.4 Hz, 5-H), 5.95 (1 H, m, alkenic), 7.38–7.48 (4 H, m, NH + 3 H + 2 Ar-H), 7.56 (1 H, t, *J* 6.7 Hz, Ar-H) and 8.00 (2 H, d, *J* 7.0 Hz, Ar-H).

Acknowledgements. We are grateful to Drs. J. Ø. Madsen, J. Møller, B. H. Nielsen, H. Olsen, and C. T. Petersen for their skilful experimental assistance in various phases of this work.

References

1. Beecham, A. F. *Tetrahedron 28* (1972) 5543.
2. Legrand, M. and Rougier, M. J. In: Kagan, H. B., Ed., *Stereochemistry: Fundamentals and Methods*, Vol. 2, p. 140, and references therein, Thieme, Stuttgart 1977.
3. Pravdić, N. and Fletcher, H. G. *Carbohydr. Res. 19* (1971) 339.
4. Horton, D., Thomson, J. K., Varela, O., Nin, A. and de Lederkremer, R. M. *Carbohydr. Res. 193* (1989) 49.
5. Rollin, P. *Tetrahedron Lett. 28* (1987) 3813.
6. Harada, N. and Nakanishi, K. *Circular Dichroic Spectroscopy-Exciton Coupling in Organic and Bioorganic Chemistry*, Chap. 7-4, pp. 260–272, University Science Books, Mill Valley, CA 1983.
7. Gonnella, N. C., Nakanishi, K., Martin, V. S. and Sharpless, K. B. *J. Am. Chem. Soc. 104* (1982) 3775.
8. Lo, L.-C., Berova, N., Nakanishi, K., Schlingmann, G., Carter, G.T. and Borders, D. B. *J. Am. Chem. Soc. 114* (1992) 7371.
9. Mori, Y., Kohchi, Y., Suzuki, M. and Furukawa, H. *J. Am. Chem. Soc. 114* (1992) 3557.
10. Herzig, J. and Nudelman, A. *Carbohydr. Res. 153* (1986) 162.
11. (a) Kauzman, W., Clough, F. B. and Tobias, I. *Tetrahedron 13* (1961) 57; (b) Brewster, J. H. *J. Am. Chem. Soc. 81* (1961) 5457; *Tetrahedron 13* (1961) 106; (c) Lichtenthaler, F. W., Sakakibara, T. and Oeser, E. *Carbohydr. Res. 59* (1977) 47; (d) Liu, H. W. and Nakanishi, K. *J. Am. Chem. Soc. 103* (1981) 7005; (e) Wiesler, W. T., Berova, N., Ojika, M., Meyers, H. V., Chang, M., Zhou, P., Lo, L.-C., Niwa, M., Takeda, R. and Nakanishi, K. *Helv. Chim. Acta. 73* (1990) 509; (f) Lo, L.-C., Berova, N., Nakanishi, K., Morales, E. Q. and Vázquez, J. T. *Tetrahedron Asym. 4* (1993) 321.
12. Banfi, L., Beretta, M. G., Colombo, L., Gennari, C. and Scolastico, C. *J. Chem. Soc., Chem. Commun.* (1982) 488, and references cited therein.
13. Mukhopadhyay, T., Roy, K., Coutinho, L., Rupp, R. H., Ganguli, B. N. and Fehlhaber, H. W. *J. Antibiot. 40* (1987) 1050.
14. (a) Van Middlesworth, F., Giacobbe, R. A., Lopez, M., Garrity, G., Bland, J. A., Bartizal, K., Fromtling, R. A., Polishook, J., Zweerink, M., Edison, A. M., Rozdilsky, W., Wilson, K. E. and Monaghan, R. *J. Antibiot. 45* (1992) 861; (b) Van Middlesworth, F., Dufresne, C., Wincott, F. E., Mosley, R. T. and Wilson, K. E. *Tetrahedron Lett. 33* (1992) 297.
15. Horn, W. S., Smith, J. L., Bills, G. F., Raghoobar, S. L., Helms, G. L., Kurtz, M. B., Marrinan, J. A., Frommer, B. R., Thornton, R. A. and Mandala, S. M. *J. Antibiot. 45* (1992) 1692.
16. Banfi, L., Beretta, M. G., Colombo, L., Gennari, C. and Scolastico, C. *Gazz. Chim. Ital. 112* (1982) 51.
17. Van der Baan, J. L., Barnick, J. W. F. K. and Bickelhaupt, F. *Tetrahedron 34* (1978) 223.
18. Sangster, A. W., Thomas, S. E. and Tingling, N. L. *Tetrahedron 31* (1975) 1135.

Received July 16, 1993.